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Genetics of Novel Hybrid Bacteriophage and Development of Generalized Transducing System for Salmonella typhosa

Annual Progress Report
(From 9/1/78 to 8/31/79)

Nobuto Yamamoto, Ph.D.

August 1979

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U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND,

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Hybrids phages between Salmonella Phage P22 and coliphage and φ80 have been isolated by using E. coli-S. typhimurium hybrids. Among those hybrid phages, φ80immP22dis hybrids carrying both immunity related genes, c and Im genes of P22 were isolated. Since P22 tail component gene 9 and somatic antigen

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20. Abstract (Continued)

conversion gene al are located between the c and Im genes of P22, we examined whether ϕ80immP22dis hybrids carry these genes. Some ϕ80immP22dis hybrids carry gene al but not gene 9 whereas the remaining ϕ80immP22dis hybrids carry gene 9 only. No ϕ80immP22 hybrid phages containing both the P22 genes 9 and al were found. These observations suggest that ϕ80immP22dis hybrid is formed as a consequence of multiple crossovers.

Although λimmP22dis hybrid phages carry both genes 9 and al, ϕ80immP22dis hybrids carry only one of these genes 9 or al. Since the size of ϕ80 phage genome is about 92% of the λ genome, we concluded that the ϕ80immP22 gene is unable to contain both genes 9 and al.

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Summary

We mapped the phage chromosomes of hybrids between Salmonella phage P22 and coliphage ϕ80. Since the genomes of hybrid phages consist of clusters of genes derived from evolutionary diverse bacteriophages, we studied the genetic structure of the homology between parental phages and hybrid phages and formation mechanism of these hybrid phages. In this progress report we showed the origin of genetic segments in the hybrid phage genomes and suggested that the hybrids are formed as a consequence of multiple crossovers.

Foreword

Fundamental studies of viral genetics not only play an important role in increasing our knowledge of the action of viruses in disease processes, but have contributed greatly to our knowledge of the whole problem of cell replication, genetic transfer, gene control, morphogenesis, and antigen conversion. The significance of the study of bacterial hybrids between E. coli and Salmonella has greatly broadened with the recent discoveries of hybrid phage between coliphage and Salmonella phage. The study supported by this contract will bring many important answers for mechanisms of genetic evolution, transduction, recombination, gene expression, antigen conversion and viral replication. In addition, such newly constructed hybrids may prove useful in achieving intergeneric transduction via a hybrid phage vector, of chromosomal genes form different genera of enterobacteriace. Therefore such hybrid phages may serve as useful vectors in the genetic engineering of a polyvalent oral attenuated vaccine which expresses immunogenic determinants for antigens of Shigella, Salmonella and perhaps even cholera.

Progress

Present Status of this Project

We have previously reported the isolation of an unusal Salmonella typhimurium hybrid sensitive to coliphage λ and Salmonella phage P22 (Gemski, Baron and Yamamoto, PNAS 69, 3110, 1972). This hybrid, constructed by mating an Escherichia coli-K12 Hfr donor with an S. typhimurium recipient, was characterized as an excellent host for achieving genetic recombination between λ and P22. Two broad hybrid phage classes, each with representative types differing presumably in the extent of gene exchange, have been isolated and described in our previous reports. The λ-P22 hybrid class, which has the protein coat of λ, was found to contain at least the c region of P22. The other class, termed P22-λ, has the protein coat of Phage P22, and has inherited at least the c marker of λ.

By employing an approach similar to that previously used to isolate λ-P22 hybrids, we have been able to isolate hybrids between P22 and coliphage φ80. These newly isolated hybrid phages φ80immP22 were found to be extremely valuable phages for understanding formation mechanism of hybrids between unrelated phages.

1. Isolation and Characterization of Hybrid Phages between E. coli Phage φ80 and Salmonella Phage P22.

E. coli-S. typhimurium hybrid stain WR4027 is a rough bacterium and sensitive to coliphage φ80 for its replication but insensitive to P22 phage because of lack of P22 phage adsorption. Therefore WR4027 lysogenic for phage φ80, WR4027(φ80), is insensitive to P22 phage. By infecting WR4027(φ80) with a mixture of high titer stocks of rough specific Salmonella phages (designated R phages), we were able to isolate R-phage resistant derivatives of WR4027(φ80), designated WR4027(φ80)/R, which are smooth and fully sensitive to P22 phage. Phage P22 stocks grown on this smooth derivative of the φ80 lysogen give rise to recombinants between P22 and φ80. Such recombinants were recovered by plating on a P22 resistant host and immune to φ80, namely WR4027(φ80). They retain the protein coat of φ80 but have acquire the immC region of P22. In addition these φ80immP22 recombinant carries P22 DNA replication genes 12 and 18 as well as the x and erf genes of P22. Some φ80immP22 recombinants, designate φ80immP22dis, contain the immI region as well as the immC region, the two sidely separated loci involved in the bipartite immunity system of P22.

2. Characterization of Unusal Hybrid Phages between E. coli phage φ80 and Salmonella phage P22.

As discussed in the previous report, although λimmP22dis hybrids carry both genes 9 and al, φ80immP22dis hybrids carry gene 9 or al (Fig. 1). As shown in Fig. 2, both λ and φ80 phage genomes contain physically corresponding and functionary similar genes. These pahge genomes also carry genetically inert DNA segments which are located between their respective att and tail (J) genes. However, the entire physical length of φ80 phage genome is about 92% of the size of λ phage genome. This seems to be reflection of difference in sizes of their inert segments: φ80 carries an inert DNA segment smaller than that of λ (Fig. 2). Since the inert segments can be replaced by genes 9 and al to form dis hybrid phages,

we concluded that the φ80immP22dis hybrid phages are unable to accomodate both genes 9 and a1 simutaneously.

3. Attempts to Isolate Hybrid Phages between P22 and Mutator Phage Mμ-1.

Numerous attempts to isolate hybrids between P22 and coli mutator phage Mμ-1 were unsuccessful. This may be due to lack of induction of Mμ-1 prophage by P22 superinfection although we found that P22 infection of λ or φ80 lysogens results in induction of their prophages. Dr. Martha Howe supplied us with temperature inducible (ts) mutants of Mμ-1 phage. We shall study P22 infection of WR4027 strains lysogenic for these Mμ-1 mutants.

Future Research Plan

1. Since we isolated various φ80immP22 recombinants, we anticipate finding of P22immφ80 recombinants, which carry the early regions, at least the c region, of φ80 and retain the P22 protein coat.

2. We shall study electron microscopic heteroduplex analyses of φ80immP22 hybrid DNA with φ80 DNA. This study is under progress.

3. We shall also look for recombinants between Salmonella phage P22 and E. coli mutator phage Mμ-1.

Publications

Yamamoto, N. Wohlhieter, J.A., Gemski, P. and Baron, L.S. λimmP22dis: A hybrid coliphage λ with both immunity region of Salmonella phage P22, Molecular General Genetics, 166, 233-243, 1978.

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Yamamoto, N., Gemski, P. and Baron, L.S. Salmonella somatic O-1 antigen conversion in E. coli K12 carrying Salmonella smooth O-repeating units by λimmP22dis hybrid phages. in preparation.

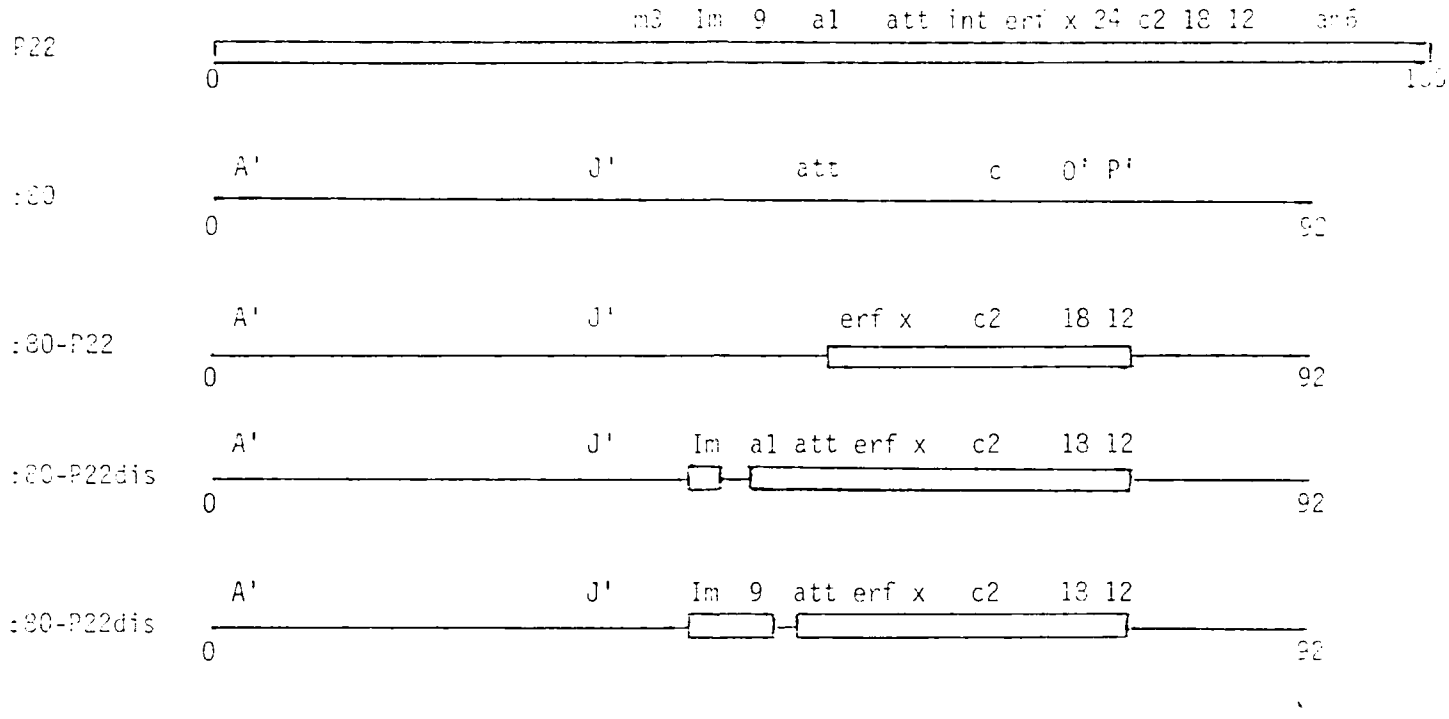

Figure 1. Genome structures of hybrids between Salmonella phage P22 and coliphage :80.

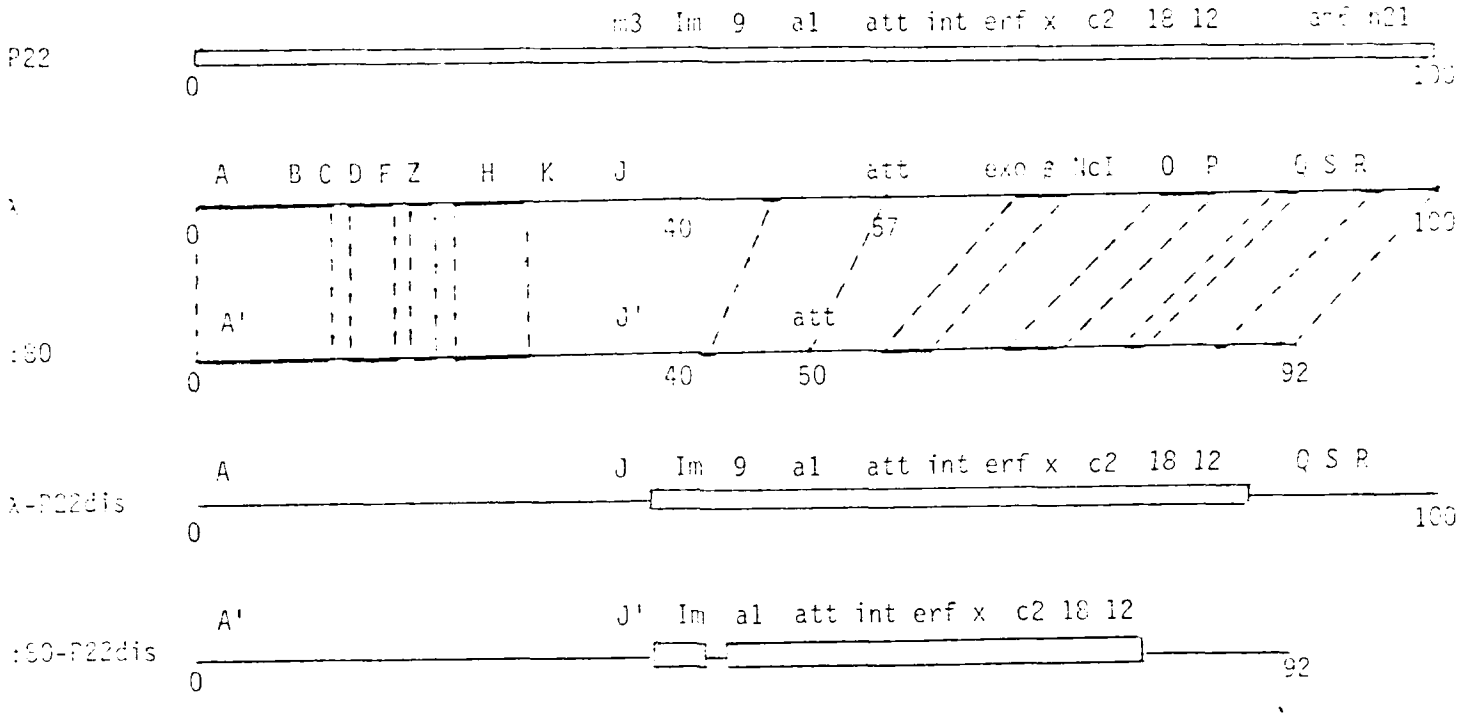

Figure 2. Genomic structures of P22, λ, :80, λ-P22dis and :80-P22dis phages.

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